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Transfections of poly(I:C), DNA, miRNA mimics and Antagomirs

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An abbreviated version of this protocol was published in eLIFE in Apr 2019

MicroRNA-deficient mouse embryonic stem cells acquire a functional interferon response

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Detailed protocol

Transfections of poly(I:C), DNA, miRNA mimics and Antagomirs
Additions to published protocol are in bold

To activate the IFN response, cells were transfected with either the dsRNA analogue poly(I:C) (Invivogen) or the Y-shaped-DNA cGAS agonist (G3-YSD Invivogen) using Lipofectamine 2000 (ThermoFisher). Transfections were performed in 24-well format, with cells approximately 80% confluent, using different concentrations of poly(I:C), from 0,5 to 2,5 µg per well (as indicated in the figures) or 0.5 µg of G3-YSD. **For each well of a 24 well plate mix 50 µl DMEM (without any additives, opti-MEM is also fine) with the desired amount of PolyIC or DNA. In our case we prepare a stock solution of PolyIC in PBS at a concentration of 1 µg/µl and would add 2.5 µl of this to 50 µl of DMEM for a concentration of 2.5 µg/well. At the same time we mix 50 µl DMEM with 1.5 µl Lipofectamine 2000 for each well of a 24 well plate. After a 5 minute incubation at RT mix 50 µl of the PolyIC or DNA mix with 50 µl of the DMEM/Lipofectamine mix and mix well. Leave for another 10 minutes at RT and add 100 µl of this mix to your 24 well plate containing 500 µl of complete medium. The medium can be replaced after 4 hours of incubation, but for many cell lines this is not necessary.** Cells were incubated for approximately 16 hr for poly(I:C)- and 8 hr for DNA-transfections before harvest and further processing. IFN-β expression was measured using a quantitative ELISA kit (Mouse IFN-β, Quantikine, R and D systems) according to manufacturer's instructions. Cells were transfected with 2.5 µg/ml poly(I:C), incubated for 16 hr after which supernatant was collected and assayed for IFN-β. To activate ESCs with exogenous IFN-β (R and D systems), cells were incubated with 10.000 U/ml of IFN-β for 4 hr, followed by RNA extraction and quantitative RT-PCR.
For the miRNA mimics (miScript, Qiagen) a final concentration of 1 µM was transfected into cells using Dharmafect (Dharmacon), incubated for the desired period and further processed. The same procedure was followed for the antagomirs (Dharmacon), but at a concentration of 100 nM. All experiments were performed in 24-well format, with cells at approximately 80% confluency. **The same amounts of DMEM and lipofectamine as described above were used for the transfection of mimics and antagomirs. If possible, it is advisable to do a titrations of your mimics/antagomirs as knock down- and transfection efficiency differs greatly between cell lines and mimics/antagomirs.**

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Witteveldt, J. and Macias-Ribela, S. (2022). Transfections of poly(I:C), DNA, miRNA mimics and Antagomirs. Bio-protocol Preprint. bio-protocol.org/prep1688.
2. Witteveldt, J., Knol, L. I. and Macias, S.(2019). MicroRNA-deficient mouse embryonic stem cells acquire a functional interferon response. eLIFE. DOI: 10.7554/eLife.44171